of the LQTS candidate genes. On the other hand, Jervell and Lange-Nielsen syndrome, which is inherited in an autosomal recessive fashion, is very rare,[8] affecting less than 1% of LQTS cases. It is caused by homozygous or compound heterozygous mutations of *KCNQ1* or *KCNE1*.[9,10]

Genetic analysis sometimes reveals two or more mutations in LQTS patients with clinical phenotypes of Romano-Ward syndrome. These compound mutations were shown to be associated with an increased arrhythmic risk.[11,12] However, most previous studies were conducted in Caucasian patients, and few systematic studies have involved Asian cohorts. In the present study, we analyzed the clinical characteristics of LQTS patients who were registered in a Japanese multicenter study. Analysis of the more 600 genotyped patients revealed that LQTS patients with compound mutations not only were common in Japan (8.4% among probands) but were associated with longer QTc and earlier onset of cardiac events. In patients who initially are diagnosed as LQT1 or LQT2, additional mutations may be present if patients have a more severe phenotype than expected; therefore, conducting a survey for major LQTS-related genes is critically important.

Methods

Patients and data collection

Major candidate genes were analyzed in 612 consecutive and unrelated probands with a suspected clinical diagnosis of congenital LQTS, who were referred to four centers in Japan (Shiga University of Medical Science, Otsu; Kyoto University Graduate School of Medicine, Kyoto; Kanazawa University Graduate School of Medical Science, Kanazawa; and National Cardiovascular Center, Suita) between June 1996 and January 2009. If gene mutations in LQTS-related genes were identified, further genetic analysis was conducted among family members as extensively as possible. All patients in the cohort were Japanese.

Genetic analysis

Informed consent was obtained from all individuals or their guardians according to standards established by the local institutional review boards. Genotypic and DNA sequence analyses of *KCNQ1, KCNH2, SCN5A, KCNE1,* and *KCNE2* were performed as described previously.[13] In addition, *KCNJ2* (Andersen syndrome [LQT7][14,15]) was analyzed in patients who had not only QT prolongation but also the clinical phenotype of Andersen syndrome, for example, periodic paralysis or dysmorphic features. Other candidate genes (e.g., ankyrin-B [LQT4], *CACNA1C* [Timothy syndrome, LQT8]) were not analyzed because mutations in these genes are extremely rare. Denaturing high-performance liquid chromatography was performed as described previously.[16] Abnormal conformers were amplified by polymerase chain reaction and sequenced using an ABI PRISM310 DNA sequencer (Perkin-Elmer Applied Biosystems, Wellesley, MA, USA). "Splicing error" mutations were defined as those that occurred within three bases of the splicing sites. When mutations were detected, 200 Japanese control subjects were checked and single nucleotide polymorphisms were excluded from the study. If mutations of these genes were detected in the probands, their family members were also analyzed and genotype–phenotype correlations confirmed. Mutation-negative controls were defined as family members without mutations detected in each proband. Nonsynonymous as well as synonymous single nucleotide polymorphisms were excluded with the assistance of data from previous reports[17–19] and from the National Center for Biotechnology Information database.

Clinical characterization

Baseline clinical data were recorded for each patient and included the following: age at diagnosis, age at first cardiac event, sex, cardiac events, family history of sudden cardiac death or LQTS members, ECG measurements, and therapeutic regimens administered. Schwartz scores also were calculated.[20,21] In the analysis of triggers of arrhythmic events, triggers were divided into four categories: exercise/swimming, emotional stress/arousal stress, sleep/rest, and other conditions.

ECG parameters measured at baseline included RR, QT_{end}, QT_{peak}, and $T_{peak\text{-}end}$ ($QT_{end\text{-}peak}$) intervals. The latter is thought to reflect transmural dispersion of ventricular repolarization.[22] Measurements were the mean of at least three beats measured in lead V_5 from the 12-lead ECG during stable sinus rhythm and corrected by the Bazett formula.[23] QT_{end} was manually measured as the time interval between QRS onset (Q) and the point at which the isoelectric line intersected a tangential line drawn at the maximal downslope of the positive T wave or the maximal

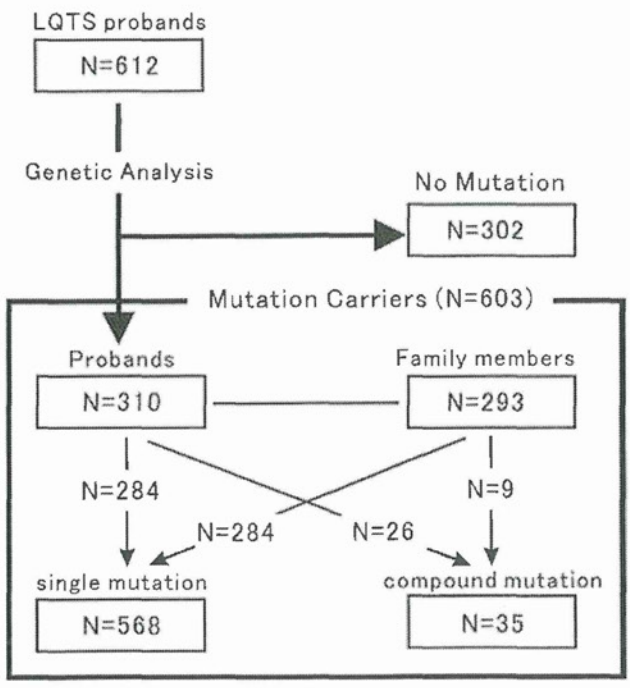

Figure 1 Schematic representation of the positive-mutation carriers in this study. LQTS = long QT syndrome.

Table 1 Overall data of patients with compound mutations

Research groups	Schwartz et al.	Westenkow et al.	Tester et al.	This study
Reported years	2003	2004	2005	2010
The corresponding number in the reference list	25	11	12	
Percentage of probands with compound mutations (probands with compound mutations/total probands) subtypes	4.6% (6/130)	5.2% (9/172*)	10.8% (29/269)	8.4% (26/310)
LQT1	7 (58%)	14 (35%)	30 (52%)	18 (35%)
LQT2	2 (17%)	10 (25%)	15 (26%)	17 (33%)
LQT3	3 (25%)	2 (5%)	13 (22%)	14 (27%)
LQT5-D85N	0 (0%)	10 (25%)	0 (0%)	0 (0%)
vs. single mutation carriers				
QTc interval	NA	prolonged	not significant	prolonged
Cardiac events	NA	frequent	not significant	not significant
Age of onset	NA	NA	younger onset	younger onset

*This table excluded probands with single nucleotide polymorphisms (SNP), NA = not available.

upslope of the negative T wave (QT_{end}). $QT_{end\text{-}peak}$ then was obtained by calculating as QT_{end} minus QT_{peak}.

Statistical analysis

All analyses were performed using the SPSS 16.0 statistical package (SPSS, Inc., Chicago, IL, USA). Data are expressed as mean ± SD. $P < 0.05$ was considered significant. Univariate comparison of parameters between groups was performed by an unpaired t-test. Differences in incidence between groups were analyzed by Chi-square test or Fisher exact probability test. The cumulative probability of a first cardiac event (syncope, torsades de pointes, ventricular fibrillation, cardiac arrest, or sudden death) occurring before age 40 years and before beta-blocker therapy or after beta-blocker therapy was determined by means of the life-table method of Kaplan-Meier, and results were compared using log rank test.[24]

Results

Genetic characteristics of mutations associated with single and compound mutations

Genetic analysis revealed gene mutations in 310 (51%) of 612 probands. The study enrolled 603 genotyped LQTS patients consisting of 310 genotyped probands and their 293 genotyped family members. A flowchart of the genetic diagnosis of the study population is shown in Figure 1.

Of the 310 genotyped probands, 26 (8.4%) had compound mutations. This rate is comparable to the rates in previous reports of Caucasian patients (Table 1). The 26 probands all had two mutations in the LQTS-related genes we examined. These 52 mutations in 26 probands consisted of 45 missense mutations, 4 frameshift mutations, 2 splice-site mutations, and 1 nonsense mutation (see Online Supplemental Data 1). The mutation types of the 284 single mutation carriers were 210 missense mutations, 34 frameshift mutations, 18 splicesite mutations, 12 deletions, 9 nonsense mutations, and 1 insertion mutation (see Online Supplemental Data 2). Therefore, the mutation types were similar between the two groups (Figure 2).

Among the 293 genotyped family members, there were 284 single mutation carriers and 9 compound mutation carriers. In total, 568 patients with a single mutation (284 probands and 284 family members) consisted of 256 with LQT1, 248 with LQT2, 62 with LQT3, and 2 with LQT5. Thirty-five compound mutation carriers (26 probands and 9 family members) consisted of 9 with LQT2 and LQT3, 7 with LQT1 and LQT2, 6 with LQT1 and LQT3, 4 with double LQT1, 3 with double LQT2 mutations, 2 with LQT1 and LQT7, 2 with LQT2 and LQT7, 1 with double LQT3, and 1 with LQT1 and LQT6.

Families associated with compound mutations

In the analysis of family members associated with compound mutations, 28 single heterozygous mutation carriers and 4 obligate single mutation carriers were identified from 9 families, and single mutation carriers had milder clinical phenotypes than compound mutation carriers (Figure 3). Only 2 (6%) of the 32 single mutation carriers had syncope but no torsades de pointes, an incidence lower than that in compound mutation carriers (54% [19/35] patients, $P < .001$). For single heterozygous mutation carriers in compound mutation families, average QTc interval was 442 ± 30 ms, which was longer than that of the 15 mutation-negative controls (408 ± 28 ms, $P = .001$) but significantly shorter than that of compound mutation carriers (510 ± 56 ms, $P < .001$).

Early onset of cardiac events and more severe QT prolongation was observed in patients with compound mutations

Table 2 compares the clinical characteristics of 35 LQTS patients with compound mutation and 568 LQTS patients with a single mutation. The female-to-male ratio was similar between the two groups. However, the incidence of family members associated with double-hit patients was significantly smaller than that with a single mutation (26% vs 50%, $P = .005$). In the ECG analysis of 496 patients with available information, corrected QT interval was significantly longer in compound mutation carriers than in single mutation carriers (510 ± 56 ms vs 478 ± 53 ms, respectively, $P = .001$), whereas other ECG findings, R-R interval, corrected QT_{peak}, corrected $QT_{peak\text{-}end}$, and rates of

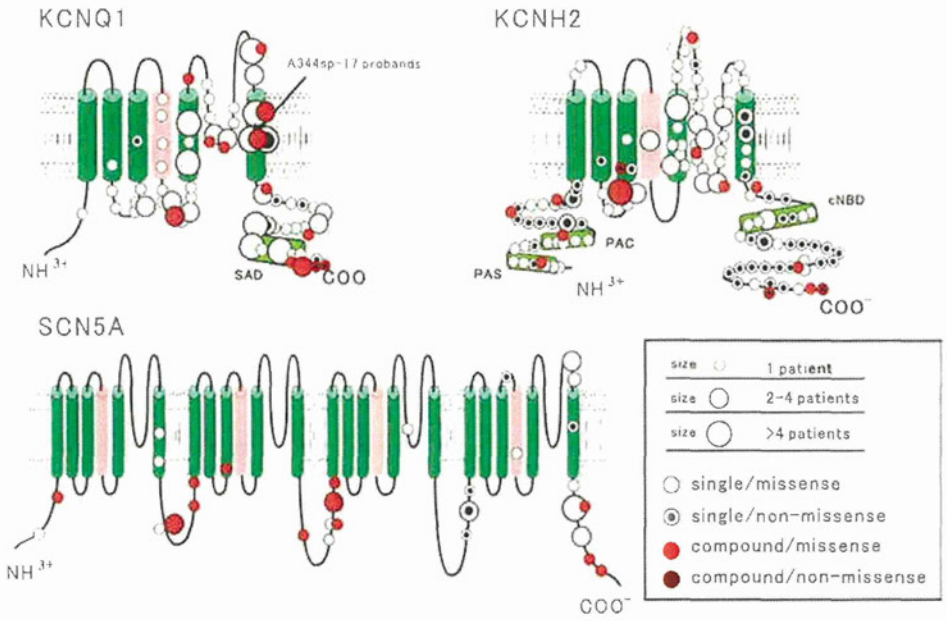

Figure 2 Conventional transmembrane topology of all mutations in the probands.

notched T wave and T-wave alternans were not different between the two groups. The frequency of patients with a normal QTc interval <440 ms was similar between the two groups, whereas the frequency of double-hit patients with QTc intervals >500 ms was significantly higher than in those with a single mutation (66% vs 26%, $P < .001$). Schwartz scores in the compound mutation group and the rate of patients with a score ≥4 were higher than those in the single mutation group (Schwartz score: 4.3 ± 2.1 vs 3.4 ± 1.9 points, $P = .017$; rates of Schwartz score ≥4 points: 70% vs 47%, $P = .026$). A significantly higher number of patients with compound mutations received beta-blocker therapy than did those with a single mutation (56% vs 33%, $P = .006$).

In the analysis of "all age groups," the frequency of cardiac events was similar between compound and single mutation groups, whereas age at first cardiac event was significantly lower in the compound mutation group (10 ± 8 years vs 18 ± 16 years, $P = .043$). For the occurrence of syncope or torsades de pointes before age 40 years, compound mutation carriers had significantly more events than did single mutation carriers (54% vs 37%, $P = .043$). The occurrence of cardiac arrest or ventricular fibrillation was similar between the two groups for patients before age 40 years. In 561 patients with available information on age at first cardiac events, Kaplan-Meier analysis showed that the cumulative rate of survival without a cardiac event before age 40 years and use of beta-blocker therapy differed significantly between compound and single mutation carriers ($P = .004$ by log rank test; Figure 4A) and between compound mutation carriers and each subgroup of single mutation carriers ($P = .004$ vs LQT1, $P = .018$ vs LQT2, $P = .001$ vs LQT3, by log rank test; Figure 4B). In the analysis of matched subtypes between single and compound mutation carriers, patients with additional mutations in an LQTS subtype had a significantly poorer prognosis than LQT1 alone ($P = .001$; Figure 5) and LQT2 alone ($P = .035$) but not LQT3 alone ($P = .06$).

Discussion

In this multicenter study, the major findings were as follows. (1) LQTS-associated compound mutations in the Japanese population were as common as previously reported in studies of Caucasian patient cohorts. (2) Patients with compound mutations displayed longer QTc and earlier onset of cardiac events. (3) Patients with compound mutations had more cardiac events before age 40 years and more beta-blocker therapy. (4) Subgroup analysis showed more cardiac events in LQT1 and LQT2 compound mutations compared to single LQT1 and LQT2 mutations.

Twenty-six probands (8.4% of genotyped LQTS) were found to have two variants in genes encoding ion channels (*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2,* or *KCNJ2*). This incidence rate is in general agreement with other studies that reported a prevalence of compound or multiple mutations of 5% to 11% of genotyped LQTS (Table 1).[11,18,25]

Table 1 summarizes the genetic and clinical characteristics of patients enrolled in previous studies and compares them with the characteristics of patients enrolled in the present study. Sanguinetti and colleagues reported that patients with compound mutations not only had longer QT intervals than single mutation carriers but also had more frequent cardiac events.[11] However, Ackerman and colleagues demonstrated that, although compound mutation carriers were diagnosed at a younger age than single mutation carriers, they did not have significantly longer QT intervals.[12] The difference between these results might be explained by half of the 20 compound probands in the cohort of Sanguinetti et al possessing the common *KCNE1-*

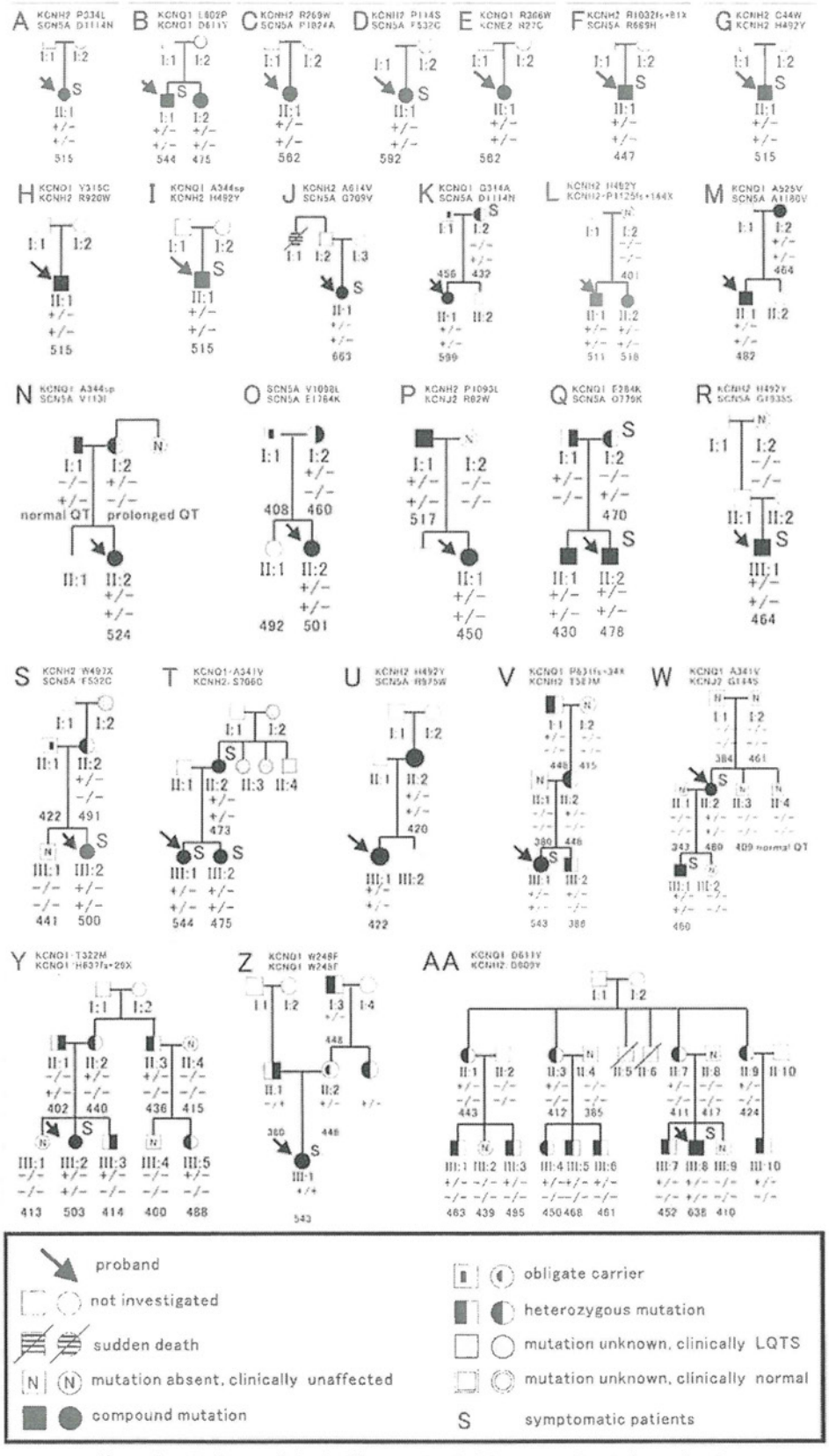

Figure 3 Pedigrees of the families associated with compound mutation probands.

Table 2 Clinical characteristics of LQTS patients with gene mutations

	Compound mutations (N=35)	Single mutations (N=568)	p value
Demographic			
Age at diagnosis (yrs)	19 ± 14 [15, 9-27]	28 ± 19 [22, 12-42]	0.001
Female gender	23 (66%)	330 (58%)	0.394
Proband	26 (74%)	284 (50%)	0.005
Family members	9 (26%)	284 (50%)	0.005
Cardiac events			
cardiac events in all age groups			
Age at first cardiac event (yrs)	10 ± 8 [11, 3.5-13.5]	18 ± 16 [12, 7-19]	0.043
syncope	19 (54%)	235 (41%)	0.161
TdP	10 (29%)	102 (18%)	0.136
cardiac arrest or VF	3 (9%)	44 (8%)	0.748
sudden death	0 (0%)	4 (1%)	1.000
cardiac events before 40 yrs			
syncope or TdP	19 (54%)	205 (37%)	0.043
cardiac arrest or VF	3 (9%)	37 (7%)	0.500
ECG measurements			
RR interval (ms)	866 ± 210	914 ± 174	0.252
corrected QT (ms)	510 ± 56	478 ± 53	0.001
corrected QT >500 ms (%)	23 (66%)	122 (26%)	<0.001
corrected QT <440 ms (%)	3 (9%)	91 (20%)	0.351
corrected QT peak (ms)	385 ± 70	384 ± 50	0.906
corrected QT peak-end (ms)	121 ± 73	95 ± 41	0.081
notched T wave	11 (31%)	200 (37%)	0.540
T-wave alternans	0 (0%)	30 (5%)	0.246
Diagnosis			
Schwartz score	4.2 ± 2.1	3.4 ± 1.9	0.017
Schwartz score ≧4	21 (70%)	219 (47%)	0.026
Therapy			
β-blocker	10 (56%)	175 (33%)	0.006
class Ib antiarrhythmic drugs	3 (9%)	53 (10%)	1.000
pacemaker	1 (3%)	15 (3%)	1.000
sympathectomy	1 (3%)	3 (1%)	0.218
defibrillator	1 (3%)	32 (6%)	0.712

TdP = torsades de pointes, VF = ventricular fibrillation, NS = not significant, corrected QT = QT interval corrected for heart rate with Bazett formula [A, B], A = median, B-C = first interquartile range-third interquartile range.

D85N polymorphism as the "second hit" (Table 1).[11,26] In all age groups of this study, the incidence of cardiac events, such as torsades de pointes or syncope, was similar between single and compound mutation carriers; however, the clinical phenotypes of those with compound mutations before 40 years of age were more serious than in those with a single mutation (Table 2). Thus, phenotypes with compound mutations appear to be more serious than single mutation carriers, regardless of race.

Beta-blocker therapy is first-line treatment for the prevention of cardiac events in LQTS. Beta-blockers have been shown to significantly reduce cardiac events in LQTS patients, especially LQT1 type.[27–29] However, patients with LQT2 or LQT3 have been reported to be less responsive to beta-blocker therapy[27,30] and may require additional therapy, such as pacemaker implantation for LQT2 or a Class Ib antiarrhythmic drug for LQT3. It may be recommended that patients with compound mutations receive additional individual therapy based on their LQTS subtype, for example, the combination of beta-blocker and Class Ib antiarrhythmic drugs for patients with LQT1 and LQT3. In patients who were first diagnosed as LQT1, Kobori et al[31] reported that additional mutations in different LQTS-related genes influenced phenotype severity and reduced beta-blocker effectiveness. Previous reports showed that approximately 20% of LQT1 patients were resistant to beta-blocker therapy. Additional or "latent" mutations may be present in these patients, and conducting a survey for major all LQTS-related genes, even after a possible mutation is identified, is critically important.

Family study analyses are of enormous importance because single mutation carriers in this study tended to have mild phenotypes. Most of the single mutation carriers in families of compound probands remained asymptomatic. However, double hits of these "latent" gene carriers could cause more serious phenotypes.[32,33] Jervell and Lange-Nielsen syndrome is a well-documented LQTS phenotype with an autosomal recessive pattern. The loss of function of I_{Ks} on both alleles generally causes not only more severe clinical phenotypes but also deafness.[9,10] In our study, two of three probands with double *KCNQ1* mutations had no deafness. We speculate that these mutations would functionally cause mild changes without complete loss of I_{Ks}. Westenskow et al[11] reported the molecular mechanism of

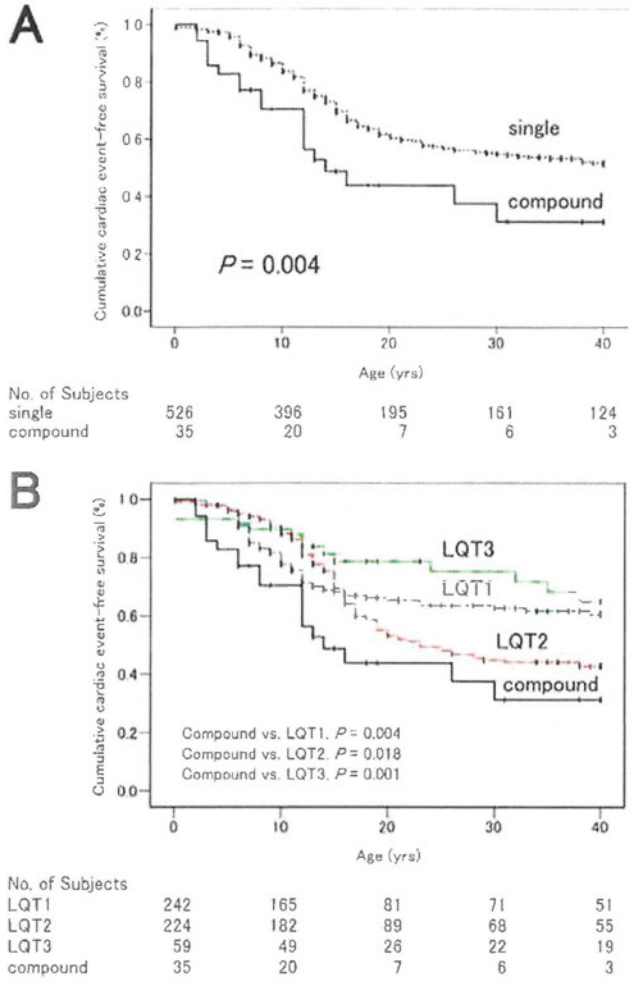

Figure 4 Kaplan-Meier cumulative probability of cardiac event-free survival from birth to age 40 years and before therapy. **A:** Comparison between patients with a single mutation and compound mutations. **B:** Comparison among patients with long QT syndrome type 1 (LQT1), type 2 (LQT2), type 3 (LQT3), and compound mutations.

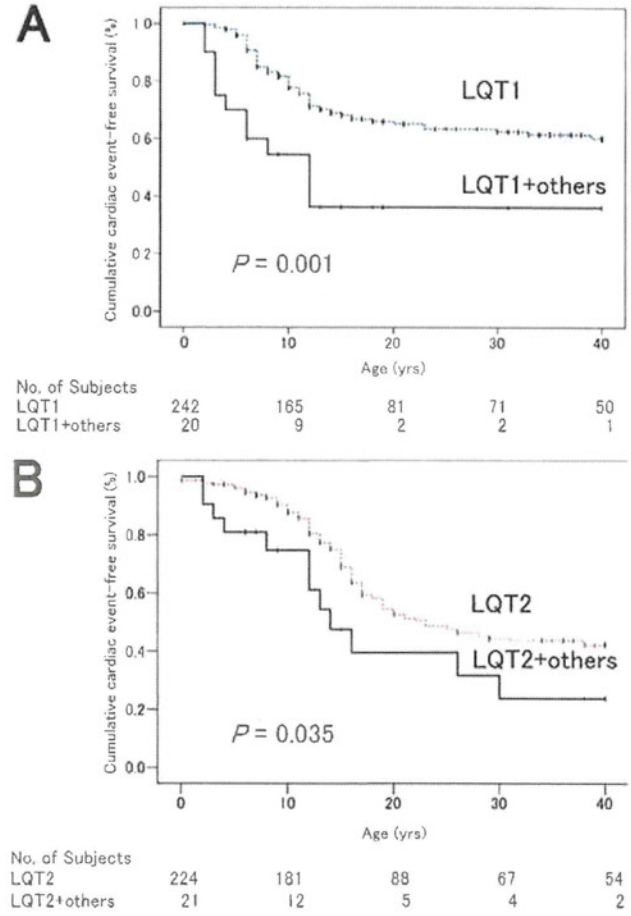

Figure 5 Kaplan-Meier cumulative probability of cardiac event-free survival from birth to 40 years of age and before therapy. **A:** Comparison between patients with long QT type 1 (LQT1_ subtype and compound mutation carriers with LQT1 plus other mutations. **B:** Comparison between patients with long QT syndrome type 2 (LQT2) and those with LQT2 plus other mutations.

increased risk through compound mutations using heterologous expressions in *Xenopus* oocytes. When wild-type and variant subunits were coexpressed in appropriate ratios to mimic the genotype of the probands with mutations, the reduction in current density was equivalent to the additive effects of the single mutations. Coexpression of two mutant subunits caused a significant but incomplete reduction. Thus, either compound mutation seems to be associated with mild functional damage. It is necessary to have "double hits" of these mild mutations in order to produce symptoms.

Study limitations

This study has several limitations. First, six major LQTS candidate genes were examined, but not for minor genes encoding a family of versatile membrane adapters. However, excluding these minor genes from our investigations would not have affected the overall study results, largely because the incidence of these minor gene mutations reportedly is ≤1%. Second, analysis of single mutation carriers in compound mutation families is dominated by their presence in only 35% (9/26) of families. Therefore, there might be a statistical bias due to a mutation-specific effect. Third, Kapa et al[19] reported the need for further studies on whether regions such as the interdomain linker of *SCN5A* could affect the clinical phenotypes of LQTS. In this study, we were able to distinguish mutations from these "genetic noises," especially in the *SCN5A* gene.

Acknowledgment

We thank Professor Pascale Guicheney (INSERM, U956, Group Hospitalier Pitié-Salpêtrière, Paris) for advice and review of the manuscript.

Appendix

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.hrthm.2010.06.013.

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ORIGINAL ARTICLE
Arrhythmia/Electrophysiology

Relationship Between Oral Amiodarone and Inappropriate Therapy From an Implantable Cardioverter Defibrillator

Takayuki Nagai, MD; Kazuhiro Satomi, MD; Takashi Noda, MD; Hideo Okamura, MD; Yuko Yamada, MD; Wataru Shimizu, MD; Kazuhiro Suyama, MD; Naohiko Aihara, MD; Shiro Kamakura, MD; Takashi Kurita, MD

Background: This study evaluated the efficacy of amiodarone for avoiding inappropriate therapies by implantable cardioverter defibrillators (ICDs).

Methods and Results: A total of 232 patients with structural heart disease (58±13 years; 78% males) who underwent an initial ICD implantation were retrospectively investigated to compare baseline characteristics and event rates of inappropriate ICD therapy delivery between patients with oral amiodarone therapy (amiodarone group, n=116) and those without (non-amiodarone group, n=116). During a mean follow-up of 29±21 months, inappropriate therapies occurred less frequently in the amiodarone group than in the non-amiodarone group (12% vs 27%, P=0.0068). As a cause of inappropriate ICD therapy, only atrial fibrillation (AF) significantly differed between the groups (3% vs 12%, P=0.01). The results of multivariate logistic regression analysis showed that amiodarone therapy (odds ratio (OR) 0.38, 95% confidence interval (CI) 0.19–0.77, P=0.0073) and no history of spontaneous AF (OR 0.27, 95%CI 0.13–0.57, P=0.0007) were independent predictors of a lower risk of inappropriate ICD therapy.

Conclusions: In the present group of ICD patients with structural heart disease, inappropriate therapy delivery occurred predominantly in those with spontaneous AF and/or without amiodarone. (*Circ J* 2010; **74:** 1302–1307)

Key Words: Amiodarone; Atrial fibrillation; Implantable cardioverter defibrillator; Inappropriate therapy

Several trials have suggested that implantable cardioverter defibrillators (ICDs) are effective not only for secondary prevention, but also for primary prevention of sudden cardiac death in patients with structural heart disease.[1–7] One of the major issues in patients receiving an ICD is the serious psychological reaction to the excessive delivery of appropriate shocks triggered by ventricular tachyarrhythmias, as well as inappropriate shocks triggered by rapidly conducted supraventricular tachyarrhythmias.[8–12] Therefore, an important rationale for adjuvant therapy with antiarrhythmic drugs in patients with an ICD is improving quality of life (QOL) by suppressing both supraventricular and ventricular tachyarrhythmias, as well as providing protection against death from arrhythmias.

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In the Optimal Pharmacological Therapy in Implantable Cardiovertor defibrillator patients (OPTIC) study, amiodarone plus β-blockers was effective for reducing the number of appropriate and inappropriate shocks from ICDs.[13] The efficacy of amiodarone for reducing inappropriate ICD therapies in patients with structural heart disease has been reported previously[14] and more recently, it was reported that amiodarone is effective for preventing inappropriate ICD therapies in selected patients with atrial fibrillation (AF).[15] However, the exact background to the reduction in inappropriate therapies provided by amiodarone has still not been fully investigated. In the present study, we assessed the efficacy of amiodarone for avoiding inappropriate therapies from ICDs and analyzed the contributing factors to a reduction in inappropriate therapies in patients with structural heart disease.

Methods

Patient Population

All patients who underwent ICD implantation with standard transvenous lead systems were included in our institutional registry from 1990 to 2005. Of 271 consecutive patients, a total of 232 patients (mean age 58±13 years, 79% males) with organic heart disease were studied. All patients had spontaneous sustained ventricular tachycardia (VT)/ventricular fibrilla-

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Table 1. Baseline Patient Characteristics

	Amiodarone group (n=116)	Non-amiodarone group (n=116)	P value
M/F	92/24	90/26	NS
Age ≥65 years	46 (40)	34 (29)	NS
Primary prevention of SCD, n (%)	7 (6)	13 (11)	NS
NYHA class III or IV, n (%)	11 (9)	4 (3)	NS
Prior pacemaker, n (%)	5 (4)	6 (5)	NS
Ischemic heart disease, n (%)	54 (47)	38 (33)	0.044
Atrial fibrillation, n (%)	26 (22)	36 (31)	NS
Paroxysmal or persistent, n (%)	18 (16)	22 (19)	NS
Permanent, n (%)	8 (7)	14 (12)	NS
Hypertension, n (%)	36 (31)	30 (26)	NS
Diabetes mellitus, n (%)	29 (25)	22 (19)	NS
LVEF, %	31±12	40±17	<0.0001
LAD, mm	43±10	40±9	0.026
Total heart beats/day	88,157±16,536	93,463±18,199	0.049
Dual chamber, n (%)	55 (47)	26 (22)	0.0001
Antiarrhythmic agents			
Class Ia, n (%)	0 (0)	8 (7)	0.0069
Class Ib, n (%)	19 (16)	32 (28)	NS
β-blocker, n (%)	65 (56)	66 (57)	NS
Sotalol, n (%)	1 (1)	9 (8)	0.019
ACEI or ARB, n (%)	74 (64)	65 (56)	NS

Data are mean±SD or number of subjects (%).
SCD, sudden cardiac death; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; LAD, left atrial diarneter; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker.

tion (VF) or a history of syncope with an inducible sustained ventricular arrhythmia.

All the patients were categorized according to the administration of amiodarone at the time of ICD implantation. Amiodarone was administered by either the referring physician or in hospital for patients who had frequent, refractory sustained ventricular arrhythmias and/or frequent premature ventricular contractions, and also in whom other antiarrhythmic drugs were not effective or tolerated. One patient with concomitant use of amiodarone and a class Ia drug was excluded from the study. The baseline characteristics and event rates of inappropriate ICD therapies between the amiodarone group and non-amiodarone group were compared. We also assessed the univariate and multivariate predictive variables of inappropriate therapies among all subjects. The mean period of follow-up was 29±21 months.

ICD Implantation and Follow-up

The ICDs were implanted by the standard transvenous approach. Dual-chamber ICD devices were selected if the patients had VT with a long cycle length (>350 ms), a supposed indication for pacemaker implantation, or a history of a supraventricular tachycardia (SVT). The presence of SVT was confirmed from the clinical chart or information obtained from the referral doctors, ambulatory ECG and ECG monitoring during hospitalization. The defibrillation threshold (DFT) was analyzed at implantation and the time of administration of the additional antiarrhythmic drugs. No patients who had received cardiac resynchronization therapy were included in this study.

After hospital discharge, the patients were followed up at the outpatient clinic every 3–6 months or immediately after any ICD shock delivery. The evaluation included a history from the patient and interrogation of the ICD for any arrhythmic events. The stored intracardiac recordings were carefully evaluated by 3 independent experienced cardiologists. ICD delivery for any atrial tachyarrhythmias, including AF, atrial flutter, atrial tachycardia or sinus tachycardia, were defined as inappropriate therapy because of SVT, which was defined by the following algorithm. Tachycardia with a regular and narrow QRS morphology on the stored intracardiac recordings retrieved from the ICD was defined as an atrial tachycardia if it had a sudden onset or no atrioventricular dissociation in patients with a dual-chamber ICD. We defined it as sinus tachycardia if the tachycardia gradually initiated at the beginning of the tachycardia. If it was an irregular tachycardia with a narrow QRS morphology, we defined it as AF.

The adverse effects of amiodarone were also evaluated. Patients were checked every 4 months at the outpatient clinic by laboratory examinations and a chest X-ray.

Statistical Analysis

The clinical outcome was the first episode of an inappropriate ICD event, including shocks or antitachycardia pacing therapy. The results are presented as percentage or the mean±SD, as appropriate. The patients with and without oral administration of amiodarone at the time of ICD implantation were compared with an unpaired Student's t-test. The categorical variables were compared using a chi-square test or Fisher's exact test. The time to the first inappropriate therapy was analyzed by the Kaplan-Meier method. Variables with $P<0.05$ in the univariate test were entered into the multivariate logistic regression analysis to identify the independent predictive variables for inappropriate therapies. The level of statistical significance was set at $P<0.05$.

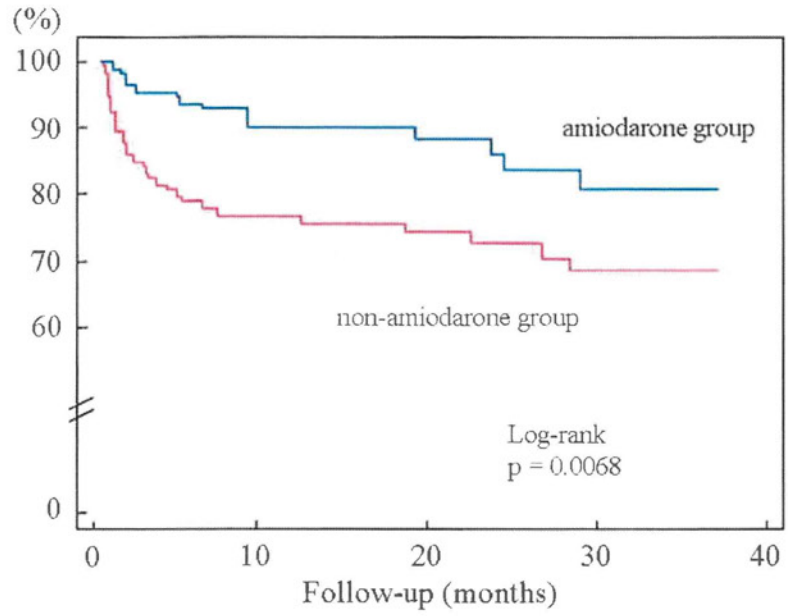

Figure. Event-free curve of inappropriate implantable cardioverter defibrillator therapy. Kaplan-Meier plots depicting the cumulative risk of inappropriate therapies. The event-free survival was better among the patients treated with amiodarone.

Table 2. Causes of Inappropriate Therapies

	Amiodarone group	Non-amiodarone group	P value
Sinus tachycardia, n (%)	6 (5)	13 (11)	NS
Atrial fibrillation, n (%)	3 (3)	14 (12)	0.0098
Atrial flutter, n (%)	1 (1)	1 (1)	NS
NSVT, n (%)	2 (2)	1 (1)	NS
Sensing failure, n (%)	1 (1)	1 (1)	NS
Lead failure, n (%)	0 (0)	1 (1)	NS
EMI, n (%)	1 (1)	0 (0)	NS
Inappropriate shock/ATP, n	10/4	16/15	NS

Data are number of subjects (%).
NSVT, nonsustained ventricular tachycardia; EMI, electromagnetic interference; ATP, antitachycardia pacing.

Results

Patient Characteristics

The baseline characteristics of the patients are summarized in **Table 1**. There were no significant differences between the 2 groups in terms of sex, age, New York Heart Association functional class, prior pacemaker implantation or history of supraventricular tachyarrhythmias. The prevalence of ischemic heart disease was higher in the amiodarone group than in the non-amiodarone group (47 vs 33%, P=0.044). The patients in the amiodarone group had a lower left ventricular ejection fraction (31±12% in the amiodarone group, 40±17% in the non-amiodarone group; P<0.0001), larger left atrial diameter (43±10 mm in the amiodarone group, 40±9 mm in the non-amiodarone group; P=0.026), and smaller total number of heart beats on the 24-h Holter monitoring (88,157±16,536 beats in amiodarone group, 93,463±18,199 beats in the non-amiodarone group; P=0.049). Dual-chamber ICDs were more frequently implanted in the patients in the amiodarone group as compared with those in the non-amiodarone group (47% in the amiodarone group, 22% in the non-amiodarone group, P=0.0001). There was no significant difference in the combined use of β-blockers. The mean daily maintenance dose of amiodarone was 170±52 mg (range, 50–400 mg) in the amiodarone group. Although no fatal side-effects were observed during the follow-up, amiodarone was discontinued because of adverse drug effects in 19 patients (16%), including thyroid dysfunction in 11 (9%), pulmonary intoxication in 6 (5%), liver dysfunction in 1 (0.9%), and proarrhythmic effects in 1 (0.9%).

Incidence and Cause of Inappropriate ICD Therapies

In total, 14 patients (12%) in the amiodarone group and 31 (27%) in the non-amiodarone group had at least 1 inappropriate therapy during a mean follow-up of 29±21 months. Event-free curves were computed for the 2 groups using the Kaplan-Meier method (**Figure**). The inappropriate therapies occurred less frequently in the amiodarone group than in the non-amiodarone group (P=0.0068 by log-rank test). The proportion of patients receiving at least 1 inappropriate shock delivery was 71% in the amiodarone group, and 52% in the non-amiodarone group. As a cause of the inappropriate therapies, only AF was significantly frequent in the non-amiodarone group (3 of 116 patients, 3% in the amiodarone group, 14 of 116 patients, 12% in the non-amiodarone group; P=0.0098) (**Table 2**). There was no history of AF documenta-

Table 3. Univariate Analysis of the Inappropriate Therapies

	Inappropriate therapy		P value
	Yes (n=45)	No (n=187)	
M/F	36/9	146/41	NS
Age ≥65 years	11 (24)	69 (37)	NS
NYHA class III or IV, n (%)	2 (4)	13 (7)	NS
Ischemic heart disease, n (%)	16 (36)	76 (41)	NS
Atrial fibrillation, n (%)			
Paroxysmal or persistent, n (%)	16 (36)	24 (13)	0.0007
Permanent, n (%)	6 (13)	16 (9)	NS
LVEF, %	38±15	35±16	NS
LAD, mm	41±9	41±10	NS
Dual chamber, n (%)	17 (38)	64 (34)	NS
Class Ia, n (%)	0 (0)	8 (4)	NS
Class Ib, n (%)	10 (22)	41 (22)	NS
Amiodarone, n (%)	14 (31)	102 (55)	0.0079
Sotalol, n (%)	2 (4)	8 (4)	NS
β-blocker, n (%)	26 (58)	105 (56)	NS

Data are number of subjects (%).
Abbreviations as in Table 1.

tion before ICD implantation in 5 of 14 patients with inappropriate therapies in the non-amiodarone group. On the other hand, all patients in the amiodarone group with inappropriate therapies caused by AF had a history of spontaneous AF before ICD implantation.

Predictors of Inappropriate ICD Therapies

The results of the univariate analysis for inappropriate ICD therapies are shown in **Table 3**. A history of spontaneous AF before ICD implantation and no administration of amiodarone were associated with an increased risk of an inappropriate therapy. The administration of β-blockers or sotalol, and dual-chamber ICD implantation did not differ statistically between those with and without inappropriate therapies. The multivariate logistic regression analysis showed that amiodarone therapy (odds ratio (OR) 0.38, 95% confidence interval (CI) 0.19–0.77, P=0.0073) and absence of preexisting spontaneous (paroxysmal/persistent) AF (OR 0.27, 95%CI 0.13–0.57, P=0.0007) remained as independent predictors of a lower risk of inappropriate therapies (**Table 4**).

Table 4. Multivariate Logistic Regression Analysis of the Inappropriate Therapies

	Odds ratio	95%CI	P value
Amiodarone	0.38	0.19–0.77	0.0073
Free from PAF	0.27	0.13–0.57	0.0007

PAF, paroxysmal or persistent atrial fibrillation; CI, confidence interval.

Discussion

Major Findings

In this study, we demonstrated that a history of paroxysmal or persistent AF was a major factor in the delivery of inappropriate therapy by an ICD in patients with structural disease. We further demonstrated that the administration of amiodarone concomitant with implantation of an ICD was associated with less inappropriate ICD deliveries.

Amiodarone as an Adjunctive Therapy With ICDs

The aim of adjunctive drug therapy with ICDs is to reduce both the number of appropriate shocks triggered by ventricular tachyarrhythmias and to prevent inappropriate shocks because of SVTs. The avoidance of frequent shocks through the use of antiarrhythmic agents may be crucial for the safety and QOL of patients with ICDs.

Amiodarone, which prolongs the action potential duration and refractoriness of cardiac tissue, has emerged as the antiarrhythmic agent of choice for treating life-threatening ventricular arrhythmias. Previous prospective randomized studies have suggested that amiodarone prevents the recurrence of VT/VF and unexpected death, and reduces the total mortality in patients with ventricular tachyarrhythmias. Recently, we reported the usefulness of amiodarone therapy guided by VT inducibility for preventing VT/VF recurrence in patients with structural heart diseases and relatively preserved left ventricular ejection function (≥30%).[16] On the other hand, the combined use of antiarrhythmic agents with ICDs might lead to adverse responses such as an unacceptable increase in the DFT, underdetection of VT/VF because of prolongation of the arrhythmia cycle length beyond the programmed detection interval or potential proarrhythmias or extracardiac toxicity. Amiodarone is widely used as an adjuvant drug therapy with ICDs; however, there have been few randomized placebo–control trials to evaluate whether amiodarone is beneficial or not.

The efficacy of combination therapy with oral amiodarone plus β-blockers in patients receiving a dual-chamber ICD for secondary prevention has been prospectively evaluated in a randomized multicenter trial,[13] which compared both amiodarone plus β-blockers and sotalol with standard β-blocker therapy for the prevention of ICD shocks in patients with an ejection fraction ≤40% and spontaneous or inducible ventricular tachyarrhythmias and confirmed the efficacy of amiodarone plus β-blockers for avoiding both excessive appropriate and inappropriate shocks without significantly increasing the risk of treatment related mortality. Recently, amiodarone alone also has been shown to be effective for avoiding inappropriate ICD therapies in a patient with AF and congestive heart failure.[14,15] Our study evaluated inappropriate ICD ther-

apies, including not only shock therapies but also antitachycardia pacing therapies, from either single- or dual-chamber ICD systems in patients with and without the oral administration of amiodarone, and our reaults suggest that not using amiodarone was associated with a higher risk of inappropriate therapies in patients with organic heart disease, regardless of β-blocker administration.

Effect of Amiodarone on Inappropriate ICD Therapies

In the present study, only AF was a significant independent cause of inappropriate therapies in a comparison of patients with and without amiodarone. The incidence of inappropriate therapies caused by AF was 2.6% and 12.1%, respectively. Interestingly, half of those patients received inappropriate therapies because of newly developed AF in patients without amiodarone. Further, no cases of inappropriate therapies caused by newly developed AF were observed in the amiodarone group. The patients implanted with an ICD usually have structural heart disease with impaired LV function. There is increased LV end-diastolic pressure caused an increased left atrial pressure from nearly 15–25% and greater complications of AF in these patients.[1,5–7] The reduction in the incidence of spontaneous AF is most likely 1 of the mechanisms of the reduction in inappropriate therapies by ICDs with adjunctive amiodarone. The effect of amiodarone in preventing newly developed AF has been widely recognized.[17,18] Singh et al showed the effectiveness of amiodarone in preventing new AF in patients with sinus rhythm at baseline and reported that ICD shocks were more often seen in patients with AF.[19] Amiodarone also has a β-blocker-like effect and decreases the heart rate during sinus rhythm or AF.[20] However, in the present study it was not possible to clarify whether amiodarone could reduce the frequency of spontaneous AF or decrease the heart rate during AF by its negative chronotropic effects.

Study Limitations

First, this was a retrospective, single-center study and lacked the clear advantage of a multicenter prospective, randomized study. The OPTIC trial suggested that amiodarone and β-antagonists could prevent inappropriate ICD therapy and our study has emphasized that result. However, β-blocker use was only 44% in a large cohort study in Japan,[21] indicating it is not frequently administered in the Japanese patient population with structural heart disease and ICD implantation. We could not confirm the reason for the relatively lower use of β-blockers in our study. One possible reason is that we included patients who underwent ICD implantation at the end of the 90 s (from 1990 to 2005) and β-blockers might be not have been frequently used during that time. Second, the patients in the amiodarone group were more likely to have decreased cardiac function and a larger left atrium. Therefore, amiodarone might have been prescribed for the sicker patients, leading to an underestimation of the drug effect. Third, the ratio of dual-chamber ICD systems was significantly higher in the amiodarone group, so it is possible that the AF-discriminating algorithms of the dual-chamber devices contributed to the reduced number of inappropriate therapies in the amiodarone group;[22] however, the ratio of dual-chamber ICDs did not statistically differ between those with and without inappropriate therapies. Fourth, there was a tendency to a higher incidence of inappropriate therapy because of sinus tachycardia in the non-amiodarone group (P<0.10). Amiodarone may also contribute to reducing sinus rate. A previous study supported that amiodarone can reduce the sinus rate.[20] In fact, patients in the amiodarone group had a smaller total number of heart beats on 24-h Holter monitoring (P=0.049) in our study. However, sinus rate did not reach statistical significance as a cause of inappropriate therapy.

Conclusions

This study demonstrated that inappropriate ICD therapies occur predominantly in patients with spontaneous AF and structural heart disease. The results also suggest that amiodarone therapy may be associated with a lower rate of inappropriate ICD therapies safely under careful observation.

Disclosure

No conflict of interest.

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The positional relationship between the coronary sinus musculature and the atrioventricular septal junction

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Aims The atrioventricular (AV) septal junction includes the coronary sinus (CS) and the compact part of the AV node and its posterior extensions. It has been recognized as the target site for ablation therapy of the AV nodal reentrant tachycardia and its variant forms. Despite the clinical significance of this region, the arrangement of the musculature in the AV septal junction, including the CS, has not fully been elucidated. We tried to explore the histological muscular diversity within the AV septal junction.

Methods and results Sixteen autopsied human hearts (seven women), mean age 59.8 years, without structural anomalies, were studied. We removed the whole AV septum, including the CS opening after the macroscopic measurements, and prepared serial sections parallel to mitral and tricuspid annuli (short-axis style) to elucidate the positional relationships between the compact AV node and the CS musculature. Out of 16 hearts, the CS musculature extended deeply into the AV septal junction in eight hearts. In the other eight hearts, the CS musculature was located above the AV septal junction. In the former group, we found that the offset of both annuli was wide (mean 3.8 ± 1.4 vs. 2.4 ± 1.1 mm), the distance between CS opening and membranous septum was long (mean 14.8 ± 1.6 vs. 12.3 ± 2.2 mm), and the CS opening level was lower and closer to the His bundle level (mean 2.8 ± 1.9 vs. 5.8 ± 2.9 mm) ($P < 0.05$).

Conclusion The deep extension of CS musculature into the AV septal junction seems to increase the tissue non-uniformity in this area.

Keywords Atrioventricular node • Coronary sinus • Histology • Atrioventricular nodal reentrant tachycardia • Atrioventricular septal junction

Introduction

The Koch's triangle is known as a landmark of the atrioventricular (AV) nodal tissue.[1] It is observed from the macroscopic endocardial view of the right atrium, and is delineated by the following three elements: the tendon of Todaro, the tricuspid valve annulus, and the coronary sinus (CS) opening. In a study, it was shown that the dimensions of the Koch's triangle show considerable diversity.[2] In addition to this diversity, the myocardial orientation beneath the endocardium has not fully been elucidated. However, the recent advances of clinical electrophysiology revealed that most of the critical regions of the AV nodal reentrant tachycardia (AVNRT) prevail around the anterior CS opening,[3] though the histological prospects of the pathway of AVNRT are still obscure.[4] Therefore, we propose a modified histological method that can provide a reference for the relation between the dimensions of Koch's triangle and the arrangement of CS musculature within the AV septal junction.

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Methods

Subjects

The present study included randomly selected 16 autopsied human hearts (9 men, 7 women) without structural anomalies or a history of significant conspicuous supraventricular arrhythmia. The mean age was 57.9 ± 18.2 years ranging between 20 and 80 years. Ten of the human subjects died of malignant diseases, the others died of a liver disease, sepsis, pneumonia, brain haemorrhage, aortic aneurysm, or gastrointestinal bleeding. None of them died of structural heart disease.

Macroscopic measurement

After fixation in 10% formalin, the posterior AV septal junction, including the opening of the CS, tricuspid valve, and mitral valve, was removed en bloc. We defined the CS as the portion that begins at the confluence of the great cardiac vein and the oblique vein (bundle) of Marshall and ends with its ostium in the right atrium (CS opening).[5] We measured each dimension in this area as follows: (a) the width of the mitral valve-tricuspid valve offset, (b) the longitudinal distance between the CS opening and membranous septum (CS-MS offset), (c) the diameter and the surface area of the CS opening, (d) the distance between the CS opening and tricuspid valve annulus (septal isthmus), and (e) the distance between CS opening and membranous septum (*Figure 1*).

Microscopic observation

Removed blocks of the hearts were conventionally processed to paraffin inclusions, and then serial sections of 7-μm thickness were made parallel to both the tricuspid and mitral valve annuli, i.e. the short-axis plane of the both ventricles. From the level of the tricuspid valve to the posterior margin of CS opening, every 10th section was stained with Masson's trichrome. We observed the distribution of the CS musculature in this area, and compared it with the dimensions of the Koch's triangle. The CS musculature was defined as the myocardial coverage of the CS.[6]

Statistical analysis

For all the study subjects, we compared heart weights, and each dimension within the Koch's triangle. The values were shown as mean ± SD. One-factor ANOVA was used for statistical comparisons between the groups. A *P*-value of <0.05 was considered statistically significant.

Results

Measurements

The mean heart weight was 334.9 ± 75.8 g, ranging between 169 and 465 g. The macroscopic measurements as described in *Figure 1* were as follows: (a) the mean width of the tricuspid valve–mitral valve offset was 3.1 ± 1.4 mm, (b) the mean longitudinal distance between the CS opening level and membranous septum level (CS–MS offset) was 4.3 ± 2.8 mm, (c) the mean surface area and diameter of the CS opening were 43.7 ± 17.3 mm^2 and 10.5 ± 2.8 mm, respectively, (d) the mean septal isthmus length was 9.9 ± 1.8 mm, (e) the mean distance between CS opening and membranous septum was 13.5 ± 2.3 mm. Detailed measurements and histological patterns of the each individual are shown in *Table 1*.

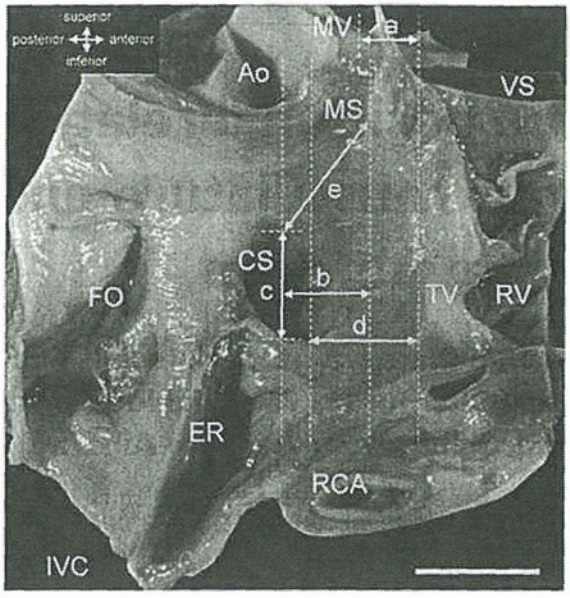

Figure 1 Macroscopic endocardial view of the Koch's triangle from the right atrium. Arrows shows the measured dimensions, (a) the width of the mirtal valve–tricuspid valve offset, (b) the longitudinal distance between the coronary sinus opening and membranous septum (CS-MS offset), (c) the diameter and the surface area of the coronary sinus opening, (d) the distance between the coronary sinus opening and tricuspid valve annulus (septal isthmus), and (e) the distance between coronary sinus opening and membranous septum. Tissue sections were sliced parallel to the dotted lines on the tricuspid valve annulus (short axis) in this photograph. Ao, aorta; CS, coronary sinus; ER, Eustachian ridge; FO, foramen ovale; IVC, inferior vena cava; MS, membranous septum; MV, mitral valve; RCA, right coronary artery; RV, right ventricle; TV, tricuspid valve; VS, ventricular septum. Bar = 10 mm.

Relationship of the distribution of coronary sinus musculature and the atrioventricular septal junction

We divided the hearts into two groups according to the distribution patterns of CS musculature.

Group A: deep extension group (*Figure 2*)

At a level just above the tricuspid valve, the CS musculature around the CS opening deeply intrudes into the AV septum and merges with the myocardium of the septal isthmus. In the middle part of the CS cavity, the CS musculature was found to have sparse and loose connections with the left atrial myocardium just beneath the compact AV node. In this group, the myocardial connections between both atria were observed mainly at the superior border of the CS opening through the CS musculature.

Group B: superficial extension group (*Figure 3*)

Sections within the septal isthmus just above both mitral and tricuspid annuli showed that the compact node was located between left and right atrial myocardium, and this configuration

Table 1 Macroscopic measurements and distribution patterns of the coronary sinus musculature into atrioventricular septum

No.	Age/sex	Heart weight (g)	a	b	c		d	e	Distribution pattern of CS musculature into AV septum
			TV-MV offset (mm)	CS-MS offset (mm)	CS opening size (mm^2)	CS opening diameter (mm)	CS-TV (mm)	CS-MS (mm)	
1	20/F	169	1.4	1	31.4	10	7	15	Group A
2	26/M	380	5.1	2	56.5	12	10	15	Group A
3	34/M	370	1.4	5	40.8	13	11	15	Group B
4	47/F	280	3.5	4	25.1	8	7	15	Group A
5	51/F	270	2.8	6	21.2	9	11	16	Group A
6	55/M	310	5.6	4	76.9	14	9	15	Group A
7	59/M	370	2.8	0	43.2	11	10	12	Group B
8	60/M	290	4.9	0	71.4	13	10	17	Group A
9	66/F	450	1.4	8	27.5	7	8	8	Group B
10	67/F	300	2.1	3	47.1	12	8	12	Group B
11	67/F	345	1.4	7	43.2	11	12	14	Group B
12	69/M	280	4.4	8	28.3	9	9	11	Group B
13	75/M	340	3.5	7	47.1	12	13	12	Group B
14	75/M	465	2.9	3	70.7	15	10	12	Group A
15	76/F	310	4.2	2	33.0	7	12	13	Group A
16	80/M	430	2.1	8	35.3	5	11	14	Group B
Mean ± SD	57.9 ± 18.2	334.9 ± 75.8	3.1 ± 1.4	4.3 ± 2.8	43.7 ± 17.3	10.5 ± 2.8	9.9 ± 1.8	13.5 ± 2.3	

AV, atrioventricular; CS, coronary sinus; MS, membranous septum; MV, mitral valve; TV, tricuspid valve; a-e, see *Figure 1*.

means that this level is above the AV septal junction and that the CS musculature does not deeply intrude into AV septum. Not only the CS musculature was found to have no connections with the left atrial myocacrdium in this level, but also myocardial connections between both atria were hardly observed.

In the middle part of the CS of this group, it was found that the musculature of the CS opening merges with the myocardium of the vestibule of the right atrium, in which it is defined as the flat endocardial sector just above the AV valve attachment (*Figure 3C*).[7] At this level, the CS musculature was close to the left atrial myocardium. The myocardial connections between both atria were observed mainly in the antero-superior border of CS opening through the CS musculature.

Comparison between the dimensions of each group

The CS musculature showed a deep extension distribution into the AV septal junction in eight hearts (Group A, *Figure 2*). The musculature around the CS opening spread beneath the level of the mitral valve annulus as shown in *Figure 2B*. The remaining eight hearts (Group B, *Figure 3*) showed that the CS musculature was located above the AV septal junction and showed non-intruding distribution, i.e. superficial extension. In Group A, the offset of both annuli was significantly wider (mean 3.8 ± 1.4 vs. 2.4 ± 1.1 mm, $P < 0.05$), the length between the CS opening and the membranous septum was longer (mean 14.8 ± 1.6 vs. 12.3 ± 2.2 mm, $P < 0.05$), and the CS opening level was lower and closer to the membranous septum level (His bundle level) (mean 2.8 ± 1.9 vs. 5.8 ± 2.9 mm, $P < 0.05$; *Table 2*).

Figure 4 represents a schematic explanation for the distributions of the CS musculature in both groups. In Group A (deep extension, *Figure 4A*), one normal variant of the tricuspid valve anatomy shows the downward displacement of the tricuspid valve annulus into the right ventricle, which draws the CS opening more anteriorly with constant distance between the tricuspid valve annulus and the CS opening. In this group, the CS is facing the compact AV node by its superior margin. This orientation brings the CS musculature to a close relation beneath the compact AV node. In Group B (superficial extension, *Figure 4B*), another tricuspid valve anatomical pattern is shown in which the tricuspid valve is straight, pushing the CS opening more posteriorly. In this group, the CS opening is facing the compact AV node by its antero-superior margin, an orientation that brings the CS musculature away from the level of the compact AV node. There was no significant difference between both groups regarding age, sex, heart weight, CS diameter, and the septal isthmus length (*Table 2*).

Discussion

The AV conduction axis begins from the top of the Koch's triangle.[1] It then penetrates the central fibrous body, and bifurcates into bundle branches beneath the membranous septum. The histological approach for the AV node has not been changed since Tawara's monograph was established.[8] In that method, the histological exploration of the AV conduction axis has mainly been

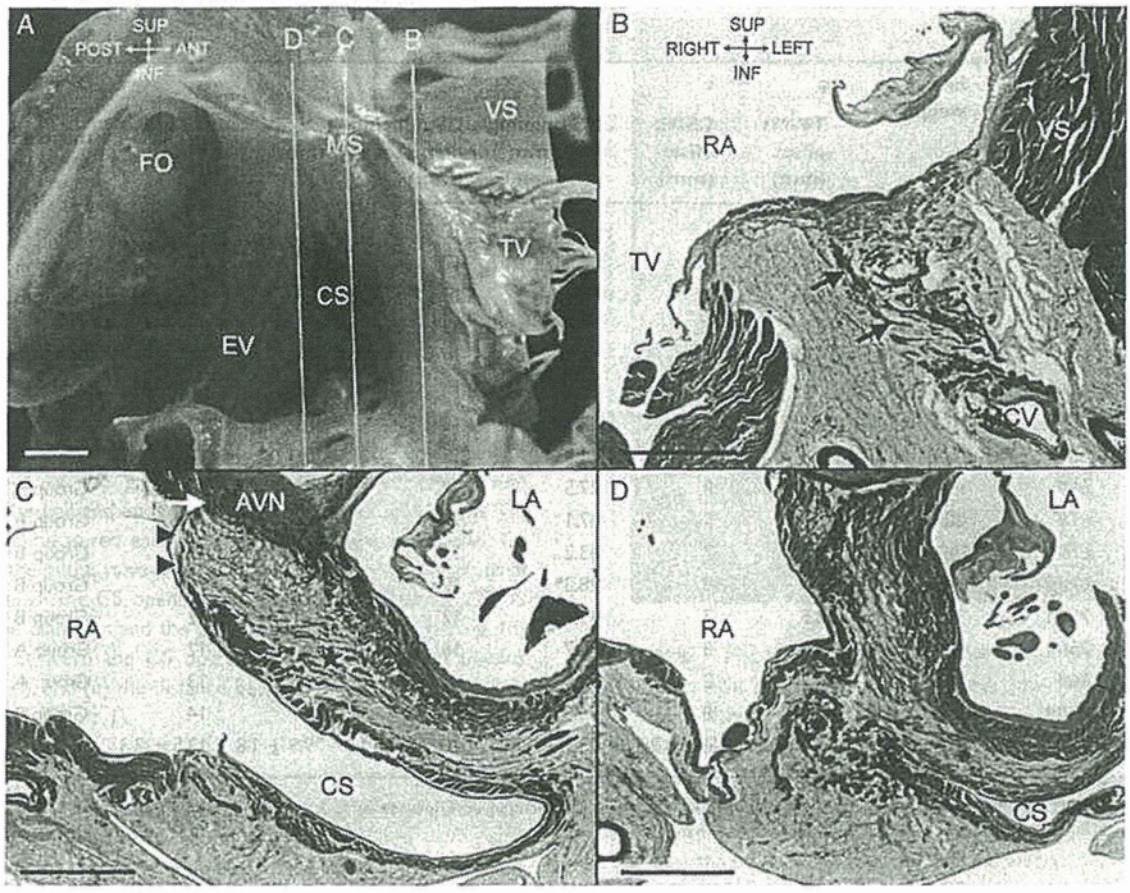

Figure 2 Representative of hearts with the Group A (the coronary sinus musculature deeply extends into the atrioventricular septum). (*A*) The macroscopic endocardial view of the Koch's triangle. Three lines (*B–D*) show the levels of histological sections. (*B*) Level B is within the septal isthmus, the coronary sinus musculature intrudes into the atrioventricular septal junction and merges with vestibule myocardium (black arrows). (*C*) Level C is through the middle part of the coronary sinus opening, the coronary sinus musculature shows loose connections with the left atrial myocardium (black star) just behind the atrioventricular node. The black arrow heads show the thin strand of transitional cells which are interposing between the atrioventricular node and the intermingled muscles of coronary sinus and vestibule. The white arrow shows the junction of the atrioventricular node and the penetrating bundle of His. (*D*) Level D is the posterior margin of the coronary sinus opening adjacent to the Eustachian ridge, the coronary sinus musculature scarcely has connections with left and right atrial myocardium. The connection between both atria through the coronary sinus musculature is observed mainly at the anterior border of the coronary sinus opening (No. 2, 26-year-old male). ANT, anterior; AVN, atrioventricular node; CS, coronary sinus; EV, Eustachian valve; FO, foramen ovale; INF, inferior; LA, left atrium; MCV, middle cardiac vein; MS, membranous septum; MV, mitral valve; POST, posterior; RA, right atrium; SUP, superior; TV, tricuspid valve; VS, ventricular septum. Bars = 5 mm.

performed by vertically sliced sections of the AV annuli, and this method has been applied for many years.[9] The CS opening is one of the elements composing the Koch's triangle.[1] However, these traditional vertically sliced sections of the Koch's triangle had technical limitations with regard to observing the histological structure of the lower part of the Koch's triangle, including CS opening due to its curvature and the divergence of both mitral and tricuspid annuli. Our histological method employed in this study, in which we made sections parallel to both annuli, provides the reference to the relation between the dimensions of Koch's triangle and the arrangement of CS musculature. Furthermore, these specimens showed both atria in the famous clinical fluoroscopical left anterior oblique (LAO) view style and made it easy to observe myocardial connections between both atria. Chauvin *et al.*[5] described the myocardial connections between the left atrial myocardium and the CS musculature using a similar method. Furthermore, Racker and Kadish[10] highlighted the myocardial arrangement within the AV node using the three-plane histological sectioning in dog hearts. However, they have not explored the myocardial arrangement around the AV septal junction. Inoue and Becker[11] described the distribution of the compact node and its extensions with reference to the substrate of the slow pathway in AVNRT in the AV septal junction. In their study, however, spatial distribution of the CS musculature around the AV nodal tissue was not mentioned. The present study with short-axis style sections unveiled the right and left atrial connection beneath the septal isthmus. This myocardial area that might participate in AVNRT was shown in a single autopsy case report.[12]

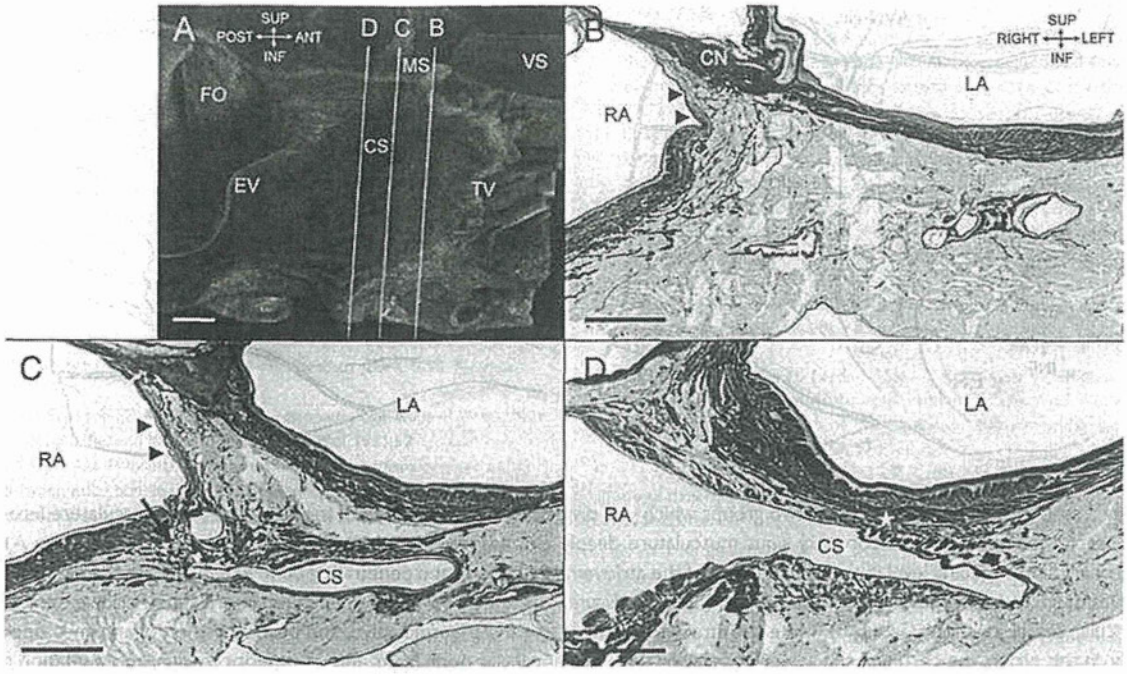

Figure 3 Representative of the hearts with Group B (the coronary sinus musculature superficially extends above the atrioventricular septal junction). (*A*) The macroscopic endocardial view of Koch's triangle. Three lines (*B–D*) show the levels of histological sections. (*B*) Level B is within the septal isthmus, myocardial connection between the both atria is hardly observed except for the compact atrioventricular node. The black arrow heads show the indirect continuity between the atrioventricular node and the vestibule muscle of the right atrium. This thin muscle strand shows the transitional cells. (*C*) Level C is the anterior part of the coronary sinus opening, the coronary sinus musculature merges within the right atrial myocardium at the antero-superior border of coronary sinus opening. The black arrow heads show the transitional cells. (*D*) Level D is through the posterior margin of coronary sinus opening, the coronary sinus musculature adheres to the left atrial myocardium (white star). (No. 9, 66-year-old female). ANT, anterior; CS, coronary sinus; CN, compact node; ER, Eustachian ridge; FO, foramen ovale; INF, inferior; LA, left atrium; MS, membranous septum; MV, mitral valve; POST, posterior; RA, right atrium; SUP, superior; TV; tricuspid valve; VS, ventricular septum. Bars = 5 mm.

Table 2 Comparison between the two groups

Distribution pattern of CS musculature into AV septum		Group A ($n = 8$)	Group B ($n = 8$)	*P*-value
Age (years)		51.3 ± 20.4	64.6 ± 13.9	n.s ($P = 0.15$)
Heart weight (g)		309.3 ± 85.9	360.6 ± 58.3	n.s. ($P = 0.18$)
a	TV-MV offset (mm)	3.8 ± 1.4	2.4 ± 1.1	$P = 0.04$
b	CS-MS offset (mm)	2.8 ± 1.9	5.8 ± 2.9	$P = 0.03$
c	CS opening size (mm^2)	48.3 ± 23.0	39.1 ± 7.8	n.s. ($P = 0.30$)
	CS-diameter (mm)	11.0 ± 2.9	10.0 ± 2.8	n.s. ($P = 0.49$)
d	CS-TV dimension (mm)	9.5 ± 1.8	10.3 ± 1.8	n.s. ($P = 0.42$)
e	CS-MS dimension (mm)	14.8 ± 1.6	12.3 ± 2.2	$P = 0.02$

AV, atrioventricular; CS, coronary sinus; MS, membranous septum; MV, mitral valve; TV, tricuspid valve; a-e, see *Figure 1*.

The concern about the exploration of the CS morphology, dilatation of the CS opening, or increase of the CS volume was reported using CS angiography or intracardiac ultrasounds in AVNRT cases.[13,14] Furthermore, DeLurgio *et al.*[15] have also reported a good correlation between CS volume and the presence of dual AV nodal physiology in case of AVNRT. For this reason, it is supposed that small morphological variations of the CS may provide some substrates of AVNRT. We could not calculate the real volume of the AV septal junction in these specimens, though the CS opening-membranous septum dimension was longer in CS deep extension group (Group A) than in the superficial extension group (Group B). We have reached the conclusion that not

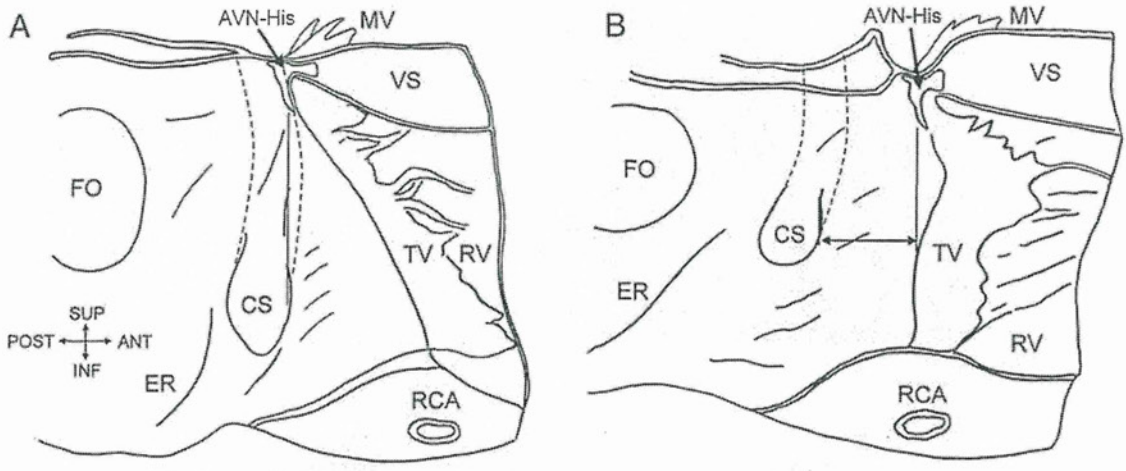

Figure 4 The schematic representation of two groups which was divided by the distribution of the coronary sinus musculature into the atrioventricular septal junction. (*A*) The coronary sinus musculature deeply extends into the atrioventricular septal junction (Group A). In these hearts, coronary sinus opening level is close to the level of the atrioventricular node and penetrating bundle of His. Note the normal downward displacement of tricuspid valve annulus into the right ventricle in *Figure 2A*. (*B*) The coronary sinus musculature superficially extends above the atrioventricular septal junction (Group B). Note the straight attachment of the tricuspid valve, and pushing the coronary sinus opening more posteriorly. In this group, the coronary sinus opening is facing the atrioventricular node by its antero-superior margin, an orientation that brings the coronary sinus musculature away from the level of the atrioventricular node. ANT, anterior; AVN, atrioventricular node; CS, coronary sinus; ER, Eustachian ridge; FO, foramen ovale; INF, inferior; LA, left atrium; MV, mitral valve; POST, posterior; RA, right atrium; RCA, right coronary artery; RV, right ventricle; SUP, superior; TV, tricuspid valve; VS, ventricular septum.

only AV nodal anatomy, but also the spatial distributions of the CS musculature, may have a role in the pathology of AVNRT, and both of them should be investigated in AVNRT cases. Furthermore, the characteristics provided by CS morphology may be of more significance in the left variant AVNRT cases, because in these cases, the ablation therapy is sometimes performed within the CS.[16]

Ho *et al.*[4] mentioned the myocardial arrangement around the compact AV node in patients with electrophysiologically proven dual AV nodal pathway using the conventional vertical histological sections. They focused on the distribution of transitional fibres approaching the AV node and found three patterns (superficial, deep, and posterior), but they concluded that the substrate of multiple pathways was ubiquitous. In our study, using the histological LAO view, both of the left and right atrial vestibules had connections with the CS musculature. In addition, the myocardial arrangements, including the CS musculature within the AV septal junction, showed non-uniformity and this phenomenon seems to be related to some of the electrical characteristics in this area.

Limitations

Because of the uncertainty in the cell-to-cell coupling under the optical microscope using the conventional staining of our study, quantification of the myocardial connections between CS musculature and the proximal AV conduction axis or the left and right atrial myocardium was not possible. We consider that using immunohistochemical staining methods, such as gap junction protein, might add information in this concern.

Although short-axis sections facilitate the histological observation of myocardial arrangements within the AV septal junction, myocardial arrangements do not always coincide with the electrophysiological phenomena. Recent experimental simulation showed that the transitional cells around the AV node and nodal extensions are associated with the formations of reentrant circuits.[17] Concerning about the participation of the CS musculature in AVNRT cases, accumulation of autopsy findings after successful ablation procedure should be required.

Conclusion

The CS location provides the variations of the muscular arrangements within the AV septal junction. Especially, the deep extension of the CS musculature into the AV septal junction seems to increase the tissue non-uniformity around the AV node.

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